REMARKS

Reconsideration of this application, as amended, is respectfully requested.

A. Claim amendments

Claims 1, 4-7, and 9-15 were pending in this application. Claims 4, 5 and 7 were cancelled without prejudice or disclaimer, new claims 17-19 were added, and claims 1, 9, 13 and 15 were amended to further clarify the invention. Support for the amendment of claims can be found in the application at paragraphs [0014], [0018] and [026] as well as Examples 3 and 4. Support for the new claims 17-19 can be found in cancelled claim 7. Claims 1, 9-15, and 17-19 are now pending in this application. No new matter has been introduced into the application as a result of the above amendment.

B. Claim Rejection under 35 U.S.C. section 112, first paragraph (New Matter)

Claim 13 was rejected for new matter. Specifically, the Examiner alleged that claim 13 included functional group limitations of diethyl aminoethyl (DEAE), carboxymethyl (CM), sulfonylmethyl (S), sulfopropyl (SP) and phosphate groups which are not supported by the original disclosure. However, the Applicants submit that, as of the priority date of this application, the abbreviations directed to the recited function groups were known. Furthermore, the Applicants submit that there is no requirement to include in the application conventional information that is well-known to a person of ordinary skill in the art at the time of filing of the application. For instance, on page 10 of "Ion Exchange Chromatography – Principles and Methods" published in 1980 has demonstrated that the functional group abbreviations for DEAE, CM and SP were known. Furthermore, on page 23 of "Ion Exchange Chromatography & Chromatofocusing" published on 2004, the abbreviation S was known. Therefore, the functional group meanings of DEAE, CM, SP, and S were well-known to the ordinary skilled artisan prior to the priority date and filing date of the instant application. Withdrawal of the new matter rejection of claim 13 under 35 U.S.C. section 112, first paragraph, is in order and is respectfully requested.

C. Claim Rejections under 35 U.S.C. section 112, first paragraph (Written Description and Scope)

Claims 1 and 3-15 were rejected under 35 U.S.C. section 112, first paragraph, for allegedly containing subject matter not described in the specification and for scope. The Examiner's comments can be found in the Office action at pages 4-9 and are not repeated here. Applicants respectfully traverse the rejection and submit that in light of the present claim amendments, the rejections are now moot.

As amended, claim 1 and its dependent claims are drawn to a method for purifying and/or isolating bacteriophage M13 using a membrane with imidodiacetic acid (IDA) charged with Cu^{+2} ions. Support can be found at paragraphs [0014], [018], [026] and Examples 3 and 4 of the specification. Furthermore, the Examples describe purification of bacteriophage M13 using the recited membrane. Accordingly, an ordinary skilled artisan in light of the teachings will understand how to make and use the claimed invention based on the teachings of the specification. Withdrawal of the 35 U.S.C. section 112, first paragraph, rejections of the aforementioned claims is in order and is respectfully requested.

D. Claim Rejection under 35 U.S.C. section 103(a)

Claims 1 and 3-15 stand rejected under 35 U.S.C. section 103(a) as being unpatentable over Sartobind membrane absorbers brochure-A("Brochure-A") in view of Fischer-Furhholz, Sartobind membrane absorbers brochure by Hirai ("Hirai"), and Rudgers et al. (Protein Enginerring, 2001, Vol. 14, pp. 487-492 ("Rudgers") as evidenced by Hondel et al. (Eur. J. Biochem., Vol. 68, pp. 55-70)("Hondel"). The Examiner alleged that it would have been obvious to one of ordinary skill in the art to apply a solution comprising bacteriophase M13 to a membrane type including Sartobind IDA (iminodiacetic acid) Cu+2 metal chelate as allegedly taught by Hirai since Brochure-A allegedly teaches membranes used in chromatography for high molecular weight biopolymers, Fischer- Frühholz allegedly teaches that Sartobind metal chelate affinity membranes have affinity to His, Cys, and Trp present in almost every protein, Rutgers allegedly teaches that M13 phase is used to display randomized peptide libraries on its surface, and Hondel allegedly teaches that M13 is a filamentous phage having a molecular weight of 1.9×10^6 Dalton. As a whole, the Examiner

believes that the claimed invention is *prima facie* obvious. The Applicants respectfully traverse this rejection.

The method of the present invention, as claimed, surprisingly allows the highly efficient isolation and/or purification of bacteriophage M13. Bacteriophage M13 is a filamentous bacteriophage with a diameter of about 6 nm and a length of about 900 nm. See the specification, for instance, at paragraph [007]. Prior to the Applicant's claimed invention, no one has thought to isolate and/or purify filamentous bacteriophages with the help of membrane-based methods since (i) filamentous bacteriophages were thought to be unable to pass the membrane structure, thus only being able to bind to the membrane surface, and (ii) filamentous bacteriophages were thought to be hard to elute from the membrane, since they easily get mechanically stuck in the membrane structure. The method of the present invention surprisingly allows for the isolation and/or purification of bacteriophage M13 at surprisingly high purities. See the specification, for instance, at paragraph [025]. Moreover, bacteriophage M13 can be isolated with a surprisingly high yield, with at least 10^{13} bacteriophages per 50 to 100 cm^2 membrane surface. See, for instance, paragraph [024]. This surprisingly high yield per membrane surface combined with a very high purity makes the method of the present invention perfectly suited for large-scale industrial applications. See the specification, for instance, at paragraph [025]. Further, isolation and/or purification of bacteriophage M13 according to the present invention is markedly improved compared to conventional purification techniques using Ni^{2+}-loaded agarose beads. See Comparative Example 1 and Figures 3A and 3B. Contrary to the Examiner's position, none of Brochure-A, Fischer- Frühholz, Hirai, and Rudgers in view of Hondel in combination teach or suggest the presently claimed invention.

Brochure-A shows *inter alia* the depletion of four different viruses that are pathogenic to mammals using ion exchange membranes. These viruses are Simian Virus 40, Reovirus type III, Murine Leukemia Virus, and Pseudorabies Virus. These viruses all have an icosaedric form and are relatively small, with a size ranging between 45 and 250 nm. In view of the relatively small icosaedric viruses used, a person of ordinary skill in the art in view of Brochure-A would expect large filamentous bacteriophages such as M13 (length about 900 nm) to bind only on the outer surface of the membrane, since it would be expected

that such large particles cannot pass through the membrane and would not be elutable as a result. As a consequence, an ordinary skilled artisan would expect, at best, that only insufficient binding capacities could be achieved, since the inner surface of the membrane would not be available for virus binding. Thus, brochure-A can be considered to teach away from purifying and/or isolating bacteriophase M13 as presently claimed.

While Brochure-A suggests that Sartobind® membranes can be used for virus purification, Brochure-A does not teach any specific examples. In particular, Brochure A neither teaches nor suggests the use of Sartobind® metal chelate membranes for the isolation and/or purification of large filamentous bacteriophages such as bacteriophage M13. Therefore, a person of ordinary skill in the art having knowledge of Brochure-A would not be motivated to make and use the claimed method for purifying and/or isolating bacteriophage M13 based on Brochure-A's teachings. As discussed below, the teachings of Fischer-Frühholz, Hirai, Rudgers and Hondel do not remedy the deficiencies in Brochure-A's teachings.

Fischer-Frühholz teaches *inter alia* a metal chelate membrane, but suggests as possible applications thereof only the purification of membrane proteins and His-tagged proteins. While Fischer-Frühholz does relate to the purification of α-Herpesvirus, Simian Virus 40, Reovirus type III, Murine Leukemia Virus, and Pseudorabies Virus using ion exchange membranes, Fischer-Frühholz, like Brochure A, neither teaches nor suggests the isolation and/or purification of any large filamentous bacteriophage such as bacteriophage M13 using a metal chelate membrane. Thus, even if Fischer-Frühholz's teachings were combined with Brochure-A, an ordinary skilled artisan will not be motivated to make and use the present invention as claimed.

Hirai merely relates to the isolation and/or purification of α-Herpesvirus, Simian Virus 40, Reovirus type III, Murine Leukemia Virus, and Pseudorabies Virus using ion exchange membranes. Hirai neither teaches nor suggests the isolation and/or purification of large filamentous viruses such as bacteriophage M13 with the help of metal chelate membranes. Moreover, Hirai specifically states that viruses of sizes ranging between 30 and 200 nm can be purified using Sartobind® membranes (cf. section "Summary", of Hirai *at al.).* Therefore, a person of ordinary skill in the art, in view of Hirai's teachings, would not

expect that purification of bacteriophage M13, having a length of about 900 nm, would be possible. Furthermore, even if Hirai's teachings were combined with Brochure-A and Fischer- Frühholz's teaches, a person of ordinary skill in the art in view of such teachings would not have been motivated to make and use the claimed invention with any reasonable expectation of success.

Rudgers and Hondel are even more remote to the presently claimed invention. Rudgers merely relates to a phage library used to find new inhibitors of TEM-1 β-lactamase via phage display. Hondel relates to the sequencing of the M13 genome. A disclosure of a phage library and M13 genome sequencing, even in combination with teachings of Brochure-A, Fischer- Frühholz, and Hirai, provides no teachings or suggestion of any method for the isolation and/or purification of bacteriophage M13. Therefore, even if the teachings of Rudgers and Hondel were combined with Brochure-A, Fischer- Frühholz, and Hirai, the resulting combined teachings would not have provided any motivation to a person of ordinary skill in the art to make and use the presently claimed invention. The combined teachings of Brochure-A, Fischer-Frühholz, Hirai, Rudgers, and Hondel does not teach or suggest a method for the isolation and/or purification of bacteriophage M13 using the recited membrane. Furthermore, the combined teachings of the cited references would not provide any motivation to a person of ordinary skill in the art to make and use the claimed invention with any expectation of success. Motivation to make and use the presently claimed method for purifying and/or isolating bacteriophage M13 using the recited membrane could only have been derived by the teachings in the applicant's specification, not from the combination of the cited references.

Accordingly, withdrawal of the 35 U.S.C. section 103(a) rejection of the claims based on Sartobind in view of Fischer- Frühholz, Hirai, and Rudgers as evidenced by Hondel is in order and is respectfully requested.

D. Conclusion

In view of the above amendments and discussion, the Applicants submit that the present claims are in condition for an allowance. A Notice of allowance is respectfully requested.

Prompt consideration and entry of this amendment prior to examination is respectfully requested. If there are any questions or comments regarding this Amendment or application, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully submitted,

Date: February 11, 2009

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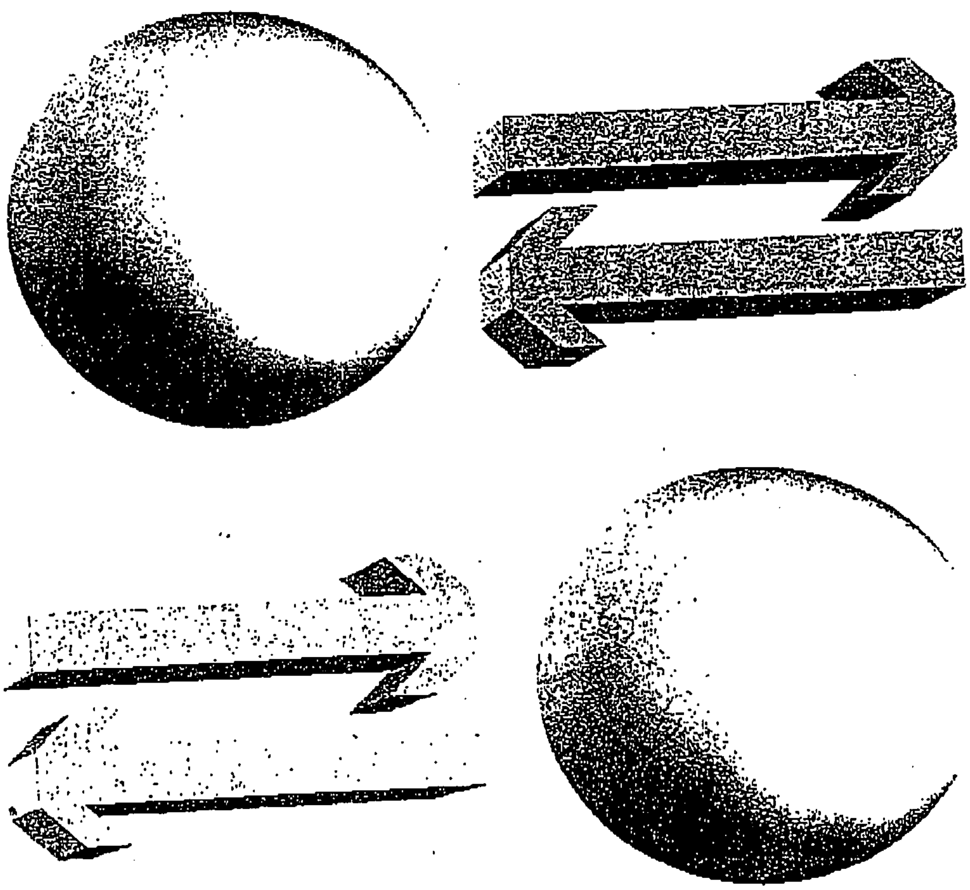
Ion Exchange Chromatography

principles and methods



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4. Sephadex ion exchangers

Sephadex ion exchangers are produced by introducing functional groups onto Sephadex, a cross-linked dextran. These groups are attached to glucose units in the matrix by stable ether linkages.

Sephadex is particularly suitable as a basis for an ion exchanger matrix. Since it is hydrophilic and shows very low non-specific adsorption, proteins, nucleic acids and other labile biological molecules are not adsorbed to or denatured by the gel. High degrees of substitution of Sephadex can be achieved without breakdown of the gel, so that high capacities are obtained. Because Sephadex is bead-formed, the gel is easy to pack and good flow properties can be obtained.

Sephadex ion exchangers are derived from either Sephadex G-25 or G-50 and swell readily in aqueous solutions. Ion exchangers based on Sephadex G-25 are more tightly cross-linked than those based on Sephadex G-50 and therefore swell less and have greater rigidity. Ion exchangers based on Sephadex G-50 are more porous than those based on Sephadex G-25 and therefore have a better capacity for molecules with molecular weights larger than 30 000. The degree of swelling of Sephadex ion exchangers depends on the pH, the ionic strength of the buffers used, and the nature of the counter-ion. It is thus not the same as that of the unsubstituted parent gel.

Four different functional groups are used for substitution onto Sephadex, giving a total of eight different ion exchangers. The functional groups are shown in Figure 1, and the resulting ion exchangers are described in Table 2.

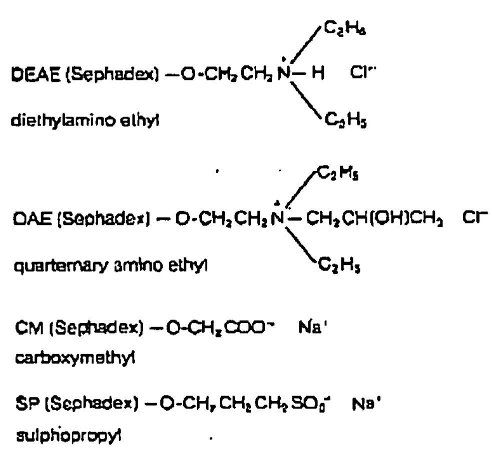

Fig. 1. Partial structures of Sephadex ion exchangers.

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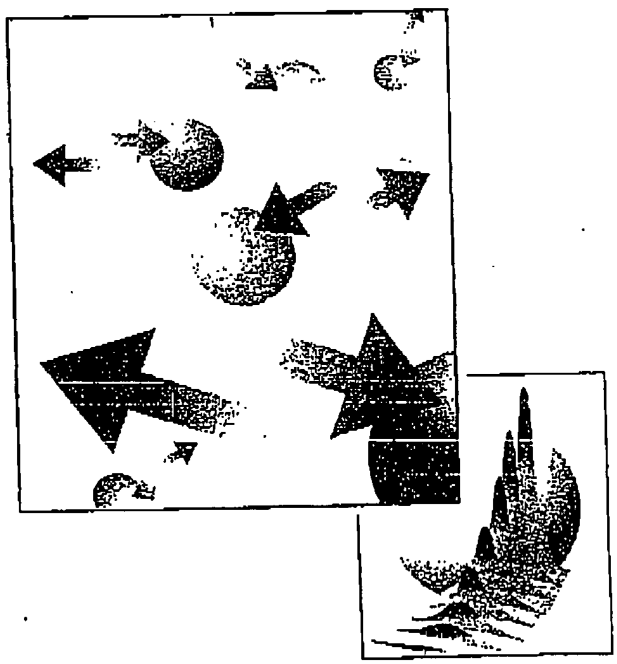
Ion Exchange Chromatography & Chromatofocusing

Principles and Methods



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Functional groups

The functional groups substituted onto a chromatographic matrix (Table 2) determine the charge of an IEX medium i.e. a positively-charged anion exchanger or a negatively-charged cation exchanger.

Table 2. Functional groups used on ion exchangers.

Anion exchangers		Functional group
Quaternary ammonium (Q)	strong	$-O-CH_2N^+(CH_3)_3$
Diethylaminoethyl (DEAE)*	weak	$-O-CH_2CH_2N^+H(CH_2CH_3)_2$
Diethylaminopropyl (ANX)*	weak	$-O-CH_2CHOHCH_2N^+H(CH_2CH_3)_2$
Cation exchangers		**Functional group**
Sulfopropyl (SP)	strong	$-O-CH_2CHOHCH_2OCH_2CH_2CH_2SO_3^-$
Methyl sulfonate (S)	strong	$-O-CH_2CHOHCH_2OCH_2CHOHCH_2SO_3^-$
Carboxymethyl (CM)	weak	$-O-CH_2COO^-$

* *The active end of the charged group is the same for DEAE and ANX. The difference between them is in the length of the carbon chain of the charged group. DEAE has a diethylaminoethyl-group bound to the agarose. ANX has a diethylaminopropyl-group attached which prevents the formation of quaternary groups, giving a different selectivity compared to DEAE.*

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The terms strong and weak refer to the *extent that the ionization state of the functional groups varies with pH*. The terms strong and weak do not refer to the strength with which the functional groups bind to proteins. Strong ion exchangers show no variation in ion exchange capacity with change in pH (Figure 13). These exchangers do not take up or lose protons with changing pH and so have no buffering capacity, remaining fully charged over a broad pH range. Strong ion exchangers include Q (anionic), S and SP (cationic).

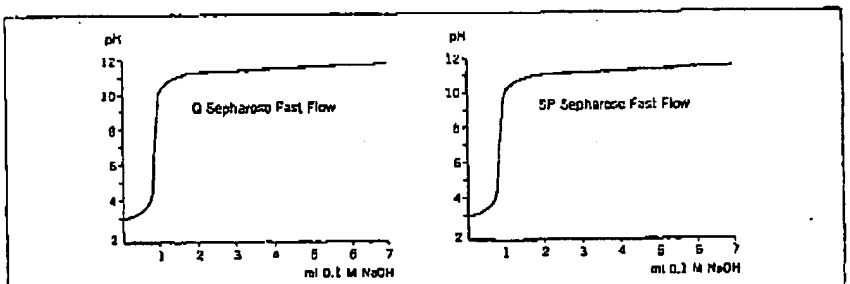

Fig. 13. Titration curves show the ion exchange capacity of strong ion exchangers Q and S. Approximately 5 ml of Q or S Sepharose Fast Flow are equilibrated in 1 M KCl and titrated with 0. 1 M NaOH.

There are several advantages to working with strong ion exchangers:

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- development and optimization of separations is fast and easy since the charge characteristics of the medium do not change with pH.
- the mechanism of interaction is simple since there are no intermediate forms of charge interaction.
- sample loading (binding) capacity is maintained at high or low pH since there is no loss of charge from the ion exchanger.

8-150, 183
8-150, 183
8-150, 183
2, 179, 180

80

7, 68,
176, 180

1, 143,

8-150, 183

0
3
0, 183

181
, 181
112,

0-82,

1, 69,
176, 180

81

, 106, 138,

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